# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Appln. No. | : | 09/973,088 | Confirmation No. 4800 |
| Applicant | : | Marie B. CONNETT-PORCEDDU | |
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| Examiner | : | Stuart F. Baum | |
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| Customer No. | : | 6449 | |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

## DECLARATION UNDER RULE 132 OF MARIE B. CONNETT-PORCEDDU

Dear Sir:

I, Marie B. Connett-Porceddu, declare as follows:

1. I am the inventor of the subject application.

2. My education and experience are as follows. I received a Bachelor of Arts degree in Biology from Humboldt State University in 1984 and a Doctorate degree in Botany from Cornell University in 1991. I have been employed by Arborgen, LLC, which is a joint venture including Westvaco Corporation, the assignee of the present application, from August, 2002 to present. I was employed as a Scientist and Senior Scientist with the role of the Mission Leader, Pine Tissue Culture and Transformation with Westvaco Corporation from 1998 to August, 2002. I was employed by Fletcher Challenge Forests from 1994 to 1997, first as a Molecular Biology Manager and then as a Biotechnology Manager. I was employed by New Zealand Forest Research Institute from 1992 to 1994 as Program Manager, Molecular Biology. I have been involved with tissue culture and transformation of pines since 1992.

3. I understand that the claims recite (a) a method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* (claims 1-9 and 11-38), (b) a method for minimizing damage to transformed cell of the pine of the genus *Pinus* subgenus *Pinus* following infection by

*Agrobacterium* (claims 39-43 and 45), (c) a method for pine cell tissue culture of pine cells of the genus *Pinus* subgenus *Pinus* (claims 46-51), (d) a method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* (claims 52-57), and (e) a method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* (claims 58-62). The method of claims 1-9 and 11-38 provides for enhanced transformation and regeneration of transformed embryogenic pine tissue in which the pine is of the genus *Pinus* subgenus *Pinus*. The remaining methods relate to various aspect of this method.

4. I have recently reviewed this application and the Office Action mailed January 28, 2003. I have also recently reviewed the Levee et al. (*Molecular Breeding* 5:429-440, 1999) reference cited in the Office Action. I have also reviewed amended claims to be filed with this Declaration.

5. I understand that the Examiner has stated that the claimed invention is unpatentable because it would have been obvious to persons skilled in the art to "use the method of Levee et al. and to optimize this method by optimization of process parameters that would not confer patentable distinction on the claimed invention." Office Action at page 4. I also understand that the Examiner has argued, with respect to Applicants' prior arguments of differences between hard and soft pines made to distinguish the invention from Levee et al., that "Applicants' mere unsupported assertions that hard pines are harder to transform than soft pines are not sufficient" Office Action at page 4. Finally, I understand that the Examiner has argued that "[n]owhere in the Levee et al reference do they mention the difference in regenerability of hard versus soft wooded pines." Office Action at page 4.

6. Applicants have invented a method which provides for enhanced transformation and regeneration of transformed embryogenic pine tissue in which the pine is of the genus *Pinus*, subgenus *Pinus*, as well as various aspects of this method. Pines of the subgenus *Pinus* are "hard pines". The method involves minimizing damage to cells subsequent to *Agrobacterium* infection and rapidly selecting transformed cells. The transformed cells are cultured to produce transgenic somatic embryos which are then germinated to produce transgenic plants. Applicants discovered that, through the use of the disclosed and claimed method and its various aspects, they were able to transform and regenerate transgenic pine plants of the *Pinus* subgenus, i.e. hard pines. Applicants'

invention allowed for the first time the transformation via *Agrobacterium* followed by regeneration of transgenic plants of pine of the subgenus *Pinus*, i.e., hard pines, especially at significant frequency.

7. Levee et al. discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, "is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*". (See page 36, first paragraph of Discussion, emphasis added). Levee et al. does not disclose the transformation and regeneration of pines of the subgenus *Pinus*. Nor would a skilled artisan expect that the method disclosed by Levee et al. for soft pines could be used or routinely modified for use with hard pines.

8. Specifically, it was well known at the time of the present invention that there were differences between soft pines and hard pines. These differences were seen in transformation and regeneration methods for soft pines and hard pines, such that there was no expectation of success with respect to the transformation and regeneration of hard or soft pines on the basis of the other. This knowledge is set forth in further detail in the following paragraphs.

9. Most classifications of *Pinus* recognize two major lineages: subgenus *Strobus* (haploxylon or soft pines, with one fibrovascular bundle in the needle) and subgenus *Pinus* (diploxylon or hard pines, with two fibrovascular bundles in the needle). This division is consistent with data from wood anatomy and secondary chemistry, and is supported in recent molecular phylogenetic studies. The genetic distance between subgenera, at least between *Pinus* and *Strobus*, may be as large as, or larger than the genetic distance between other conifer genera, e.g., between *Cedrus* and *Abies* (Price et al., 1987, *Systematic Botany*, **12**:91-97 (copy attached as Exhibit 1)), and if strict genetic criteria were used, they should perhaps be treated at generic rank. As is commonly known, hard pines are unable to interbreed with soft pines, though they can interbreed readily, if the correct timing and other conditions are provided, with other hard pine species (a seminal reference is Critchfield and Little, 1966, *Geographic distribution of the pines of the world*, USDA Forest Service Miscellaneous Publication 991, Washington, D.C. (copy attached as Exhibit 2); see also Little and Critchfield, 1969, *Subdivision of the genus Pinus (Pines)*, USDA Forest Service Miscellaneous Publication 1144, Washington, D.C. (copy attached as Exhibit 3)). Hard pines are unaffected by a

number of diseases, such as white pine blister rust, that readily infect soft pines. Their susceptibility to *Agrobacterium* infection appears to be quite different as well (personal communications from Dr. Krystyna Klimascewska and Dr. Armand Seguin, both of the Canadian Forest Service).

10. The differences between soft pines and hard pines have been shown for somatic embryogenesis of these pine subgenera. Specifically, Klimaszewska et al. (US 6,200,809 (copy attached as Exhibit 4)) demonstrates differences between soft and hard pines in the maturation of somatic embryos. Table 4 shows that number of somatic embryos and the germination percentage increased for soft pine (*Pinus strobus*) as the gellan gum content of the medium increased from 0.4% to 0.6% to 0.8% to 1.0%. Table 10, however, shows that the number of somatic embryos and germination percentage increased for hard pine (*Pinus taeda* (loblolly pine)) as the gellan gum content of the medium increased from 0.4% to 0.8% but decreased for hard pine (*Pinus taeda* (loblolly pine)) as the gellan gum content increased from 0.8% to 1.0%. The data in these tables further show that a higher concentration of ABA was used for the hard pine than was used for the soft pine and that the maximum germination achieved for hard pine was 57%, whereas the maximum germination achieved for soft pine was 92%. The knowledge and lack of expectation of success with respect to soft versus hard pines is also briefly described in the Declaration Under Rule 132 of Dr. Micahel Becwar filed in companion application Serial No. 09/973,089. (A copy of this Declaration is attached as Exhibit 5).

11. Prior to the present invention, there have been no reports of the regeneration of transgenic plants of pine of the genus *Pinus* subgenus *Pinus*. In fact any reports at all concerning regeneration of hard pines demonstrated that regeneration was not achieved. For example, Wenck et al. (1999, *Plant Mol Biol* **39**:407-416; copy attached as Exhibit 6) specifically stated that stably transformed regenerated transgenic plants, of hard pine had not been obtained, although stably transformed regenerated transgenic plants of Norway spruce (*Picea abies*) had been obtained with *Agrobacterium* transformation. See page 413, bottom of left column for the statement concerning the lack of stably transformed regenerated transgenic plants for loblolly pine, a hard pine.

12. It is noteworthy that the cited Levee et al. reference did not discuss regeneration of transgenic plants of hard pine. Hard pines are the most economic species of conifers, and loblolly pine is the most used species of hard pines. Despite this fact, Levee et al. did not work with hard

pines, but instead chose a species of soft pine. There have been no reports in the literature of the application of the method of Levee et al. to the regeneration of transgenic hard pines, either by Levee or by any other group. In addition, Levee has not continued use of the method with soft pines (personal communications from Dr. Krystyna Klimascewska and Dr. Robert Rutledge, both of the Canadian Forest Service). Finally, we have tried at Westvaco Corporation, the assignee of the present application, to use or modify for the regeneration of transgenic hard pine the method described by Levee et al. for the regeneration of a species of transgenic soft pine, but have not been successful. This lack of success with the Levee et al. method led to the present invention.

13. Experiments had been underway at Westvaco Corporation for more than 10 years to adapt systems for regenerating hard pines and for transforming and regenerating transformed hard pines. Somatic embryogenesis systems had been developed which worked well for regenerating hard pines. However, regenerating transgenic hard pines met with little or no success. Regeneration of transgenic hard pines produced by biolistic transformation procedures had been accomplished, but at only 2% efficiency. Regeneration of transgenic hard pines produced by *Agrobacterium* transformation had not been accomplished with six scientists working on the project. The inability to adapt systems developed for transgenic soft pines to transgenic hard pines is further evidence of the differences between soft pines and hard pines and the fact that there was no expectation of success in the art for using systems developed for transgenic soft pines in regenerating transgenic hard pines. This lack of success with the adaptation of other systems to regenerating transgenic hard pines led to the present invention.

14. In summary, a person of ordinary skill in the art at the time of the present invention knew that there were differences between soft pines and hard pines with respect to tissue culture, regeneration and transformation. In view of these differences and the lack of application of methods between the soft pines and the hard pines, there was no expectation of success in the art for regenerating transgenic hard pines on the basis of a single report for the regeneration of transgenic soft pines.

15. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or of any patent issued thereon.

7-14-04
Date

Marie B. Connett-Porceddu

2411-110.Rule 132 Decl A.doc

Notary July 14, 2004
Dorchester County

Janis St. Onge
Exam Exp 4-13-12